NATIONAL BREAST CANCER COALITION

ARTEMIS PROJECT

National Breast Cancer Coalition
The Breast Cancer Deadline 2020

ANNUAL MEETINGS
MARCH 8-11, 2019

I. INTRODUCTION

The National Breast Cancer Coalition (NBCC) was formed in 1991 to end breast cancer through the power of grassroots action and advocacy. Since that time, NBCC has built a strong coalition of advocates and organizations that support its mission. In 2010, NBCC launched the **Breast Cancer Deadline 2020®** campaign including a strategic plan of action set out in a blueprint that is designed to identify by 2020 the knowledge, approaches and tools needed to end breast cancer. This unprecedented campaign includes a research component, known as the **Artemis Project®**, a collaboration of researchers, advocates, and other key stakeholders who set priorities and design and implement research plans that focus on two areas:

- **Primary Prevention:** How do we stop women and men from getting breast cancer?
- **Prevention of Metastasis:** How do we stop them from dying of breast cancer?

The various reports from previous annual meetings, found at http://www.breastcancerdeadline2020.org/about-the-deadline/artemis-project.html lay out the history of the Artemis Project. This report is a summary of discussions and recommendations made at the 2019 annual Artemis meeting. This meeting included more than 30 participants including advocates and scientific experts with knowledge ranging from immunology, biophysics, and genetics, to molecular biology, and clinical oncology.

2019 ANNUAL MEETING PARTICIPANTS

Mary Helen Barcellos-Hoff, PhD, Professor, Vice Chair, Research, Director of Radiation Biology, Department of Radiation Oncology, University of California San Francisco

Rebecca Bish, PhD, Scientific Director, The Mark Foundation for Cancer Research

Frank Calzone, PhD, Vice President, Research, REMD Biotherapeutics and Biotechnology Consultant

Stacie Canan, PhD, Executive Director, Discovery and Development, Celgene Global Health

Jayanta Debnath, MD, Distinguished Professor and Chair, Department of Pathology, University of California San Francisco, Member, Helen Diller Family Comprehensive Cancer Center

Daniel Douek, MD, PhD, Chief, Human Immunology Section, Vaccine Research Center, NIAID, NIH, DHHS

Mikala Egeblad, PhD, Associate Professor and Co-chair of the Cancer Center Signal Transduction Program at Cold Spring Harbor Laboratory (CSHL)

Stephen J. Elledge, PhD, Gregor Mendel Professor of Genetics and Medicine, Department of Genetics, Harvard Medical School, Division of Genetics, Brigham and Women's Hospital

Yaniv Erlich, PhD, Chief Science Officer, Myheritage.com, Associate Professor of Computer Science and Computational Biology, Columbia University, Core Member, New York Genome Center

Silvia C. Formenti, MD, Chair, Department of Radiation Oncology, Weill Cornell Medical College, Radiation Oncologist-in-Chief, New York Presbyterian/Weill Cornell Medical Center

Cyrus Ghajar, PhD, Director, Laboratory for the Study of Metastatic Environment, PSH Program: Translational Research Program, Fred Hutchinson Cancer Research Center

Pat Haugen, BA, Advocate, Minnesota Breast Cancer Coalition

Brandy Heckman-Stoddard, PhD, MPH, Chief, Breast and Gynecologic Cancer Research Group, Division of Cancer Prevention, National Cancer Institute

Judi Hirshfield-Bartek, RN, MS, OCN, Advocate, Dr. Susan Love Research Foundation

Simon Knott, PhD, Assistant Professor, Biomedical Sciences and Associate Director, Center of Bioinformatics and Functional Genomics, Cedars-Sinai Medical Institute

Keith L. Knutson, PhD, Associate Professor, Department of Immunology, College of Medicine, Mayo Clinic, Director, Mayo Clinic Florida Cancer Research Program

Debbie Laxague, RN, Advocate, NBCC

Christopher Li, MD, PhD, Research Professor, Epidemiology Fred Hutchinson Cancer Research Center

H. Kim Lyerly, MD, FACS, George Barth Geller Professor of Cancer Research, Professor of Surgery, Professor of Pathology and Immunology, Duke University Medical Center

Anna-Laure Papa, PhD, Assistant Professor of Biomedical Engineering, The George Washington University

Ben Ho Park, MD, PhD, The Donna S. Hall Professor of Medicine, Vanderbilt University Medical Center, Co-Leader Breast Cancer Research Program, Associate Director for Translational Research, Director of Precision Oncology, Vanderbilt-Ingram Cancer Center

Michele Rakoff, Advocate, Breast Cancer Care and Research Fund

Fran Visco, JD, President, NBCC

Alana Welm, PhD, Associate Professor, Department of Oncological Sciences, University of Utah, Investigator, Huntsman Cancer Institute, Member, Cell Response and Regulation Program

SPONSOR REPRESENTATIVE

Douglas Wall, Volcano Capital and Vance Wall Foundation

MEETING SUPPORT

Brandi Felser, MBA, Chief Operating Officer-Chief of Staff, NBCC

Jaime Fornetti, PhD, Postdoctoral Researcher, Huntsman Cancer Institute, University of Utah

Giselle Hicks, MPH, Advocate, NBCC

Kayla Kirsch, MS, President, Leapfrog Consulting

Marva Lewis McKnight, The Event Professionals

Michelle Tregear, PhD, Director, Education and Training Programs, NBCC

II. BACKGROUND AND DISCUSSION

Friday evening, March 8, was set aside for introductions, background, and general scientific discussion and presentations.

The session on Prevention of Metastasis began Saturday, March 9 to Sunday, March 10, noon, followed by the session on Primary Prevention, Preventive Vaccine.

BACKGROUND PRESENTATIONS (EACH FOLLOWED BY DISCUSSION AMONG ALL PARTICIPANTS)

Review of the Vaccine Landscape (Advocate Project Update)

Debbie Laxague

Laxague presented an overview of the vaccine landscape over the past year (trials opened or registered since 3/2018). There were no immunotherapy or vaccine-based breast cancer prevention trials, and in all trials, the experimental agent is an add-on to standard therapy. Most trials are small (n=20-100), and all are either Phase 1 or 2 (many Phase 2s are randomized). Patient populations included all stages of breast cancer, but most were in the neo or post-neo adjuvant setting, and in HER2+ or triple negative breast cancer (TNBC) subtypes. The trend continues toward more immunotherapy trials that include both vaccines and checkpoint inhibitors or other immune therapies. This year there are two landscape posters with more details – one for trials with vaccines alone (plus standard therapy) and one for vaccines with a checkpoint inhibitor or other additional immune modulator (plus standard therapy). There were five to seven trials found in each category. Several of the vaccines included in the trials are ones we have seen in previous trials.

The first immunotherapy approved for breast cancer was based on the Impassion130 trial. However, participants noted many design issues with the trial, such as heterogeneity in scoring due to the PDL1 staining including both tumor and stromal cells, insufficient statistical power, and a control arm that did not receive the standard of care. And with drug's regulatory approval, concerns identified about the trial and drug may never be answered.

A copy of the vaccine landscape is available on the NBCC website: http://www.breastcancerdeadline2020.org/about-the-deadline/artemis-project.html

Updates on the Genomic Landscape of Breast Cancer, 2018-2019

Simon Knott

Knott reviewed trends in breast cancer genomics studies over the past year. Among new DNA biomarkers were a PVT1 promoter that when silenced enhances breast cancer cell competition, whole-genome doubling that predicts increased morbidity across cancer types including estrogen-receptor positive (ER+) breast cancers independently of established clinical prognostic factors, and a RET fusion gene prevalent in HER2+ breast cancer patients that when treated with a RET inhibitor resulted in a pathologic complete response in a single patient with metastatic breast cancer. Participants noted that RET inhibitors have many side effects, although the second and third generation drugs are getting better.

Many of the novel RNA biomarkers were identified by analysis of The Cancer Genome Atlas (TCGA).

TCGA data were also used to identify race-specific breast cancer biomarkers. By inferring genetic ancestry from haplotypes in the TCGA data, one study found that the genomes of African-Americans with breast cancer had higher rates of chromosomal instability, higher rates of TP53 mutations, but lower rates of PI3K pathway mutations than those with European-American ancestry. A comparative study of Nigerian breast cancer patients with TCGA data on Black and White American women found that Nigerian hormone-receptor positive (HR+) HER2- breast cancers had more TP53 and GATA3 mutations and fewer PIK3CA mutations, molecular characteristics associated with aggressive tumor biology. A study of Asian women with breast cancer found higher rates of PI3K mutations. Participants discussed the limitations of TCGA data, such as poor clinical annotation, underrepresented tumor types, and no metastatic tumor samples.

Knott also reviewed recent genomic analyses of patient response to treatment and markers of treatment resistance.

Single-cell analysis of breast cancer research identified a distinct subpopulation cluster of tumor cells shared across patients with TNBC predictive of treatment resistance and metastasis, that TNBC resistance to chemotherapy develops partly due to pre-existing resistant genotypes adaptively selected by neo-adjuvant chemotherapy treatment and partly due to transcriptional re-programming resulting in resistant phenotypes. Single-cell analysis of immune cells in breast cancer showed a large diversity of T cells in the tumor with a subset of cells expressing features of tissue memory differentiation and high levels of immune checkpoint molecules, the key targets of immune checkpoint inhibitors.

Update on T Cell Epitope Discovery Project

Steve Elledge

CD8 T cell recognition of tumors underlies the durable control of cancer observed with immunotherapies. So, understanding the antigens driving T cell function is critical to harnessing and modulating these powerful cells. However, there are many challenges for T cell antigen discovery. Elledge presented a new screening tool for detecting targets of T cells, using a cell-based approach.

He was able to detect targets of T cells as rare as 0.1% of the population. Saturation mutagenesis of the peptides was able to determine exactly what part of the peptide the T cell receptor (TCR) recognized, and also revealed unique "footprints" for different TCRs.

Elledge then tested whether targets of self-reactive TCRs could be identified. The goal was to develop a methodology to determine the targets of T cells. The hope was to identify self-antigens or neo-self-antigens for breast cancer and other cancers, and not neo-antigens.

In summary, a platform has been developed for the high-throughput identification of functional T cell targets. The targets of cytotoxic T cells can be identified directly from patients, and the targets of "revived" TCRs can be identified by sequencing. CD4 T cell targets are more difficult, but these tools can be used to identify the targets of T cells in cancer and autoimmunity.

III. ARTEMIS PROJECT ON PREVENTION OF METASTASIS

SEED GRANT UPDATES

Project Update: Enhancing Immune Recognition of Dormant Disseminated Tumor Cells (Seed Grant Update)

Cyrus Ghajar

Ghajar updated the group on the work progressing on the Artemis seed grant. (See prior Artemis reports for background and more detail.) Late recurrences account for a considerable proportion of metastatic breast cancers. Over half of ER+ breast cancer recurrence occurs after five years. DTCs have been shown to be predictive of increased risk for distant recurrence, and elimination of DTCs associated with prolonged metastasis-free survival. DTCs have been shown to be leaving earlier than the detection of the primary tumor. The perivascular niche supports bone marrow DTC growth arrest, survival, and chemo-resistance, and DTCs persist in the bone marrow despite T cell-mediated clearance of primary orthotopic tumors. Antigen-specific T cells in the bone marrow may not be killing quiescent cells, but that does not mean that they are not interacting with them or playing a role in preventing metastasis. Data show that breast tumor cells exhibit reduced HLA I expression upon dormancy induction in 3D culture, and quiescent breast tumor cells in organotypic culture exhibit down-regulated HLA-A2 expression. Participants discussed whether endocrine therapies or CDK4/6 inhibitors promote a similar dormancy phenotype in cells.

Potential barriers to surveillance of dormant DTCs include the rarity of respective cell populations, T cell trafficking, the immune evasive properties of DTCs (which may be cell cycle related), and the immune suppressive properties of the perivascular niche. The main questions are whether antigen-specific T cell receptors eliminate dormant DTCs, and whether recognition and/or killing depend on localization in the microenvironment.

Currently, Ghajar is looking at whether CAR T cells are able to eliminate dormant DTCs in an immune-competent mouse model by allowing a dormancy window to develop. The data indicate that CAR T cells do generate memory populations and persist in bone marrow two weeks post-treatment. If we can identify why CAR T cells start to swarm, we could then identify treatments that could increase swarming to the tumor cells, and hence enhance trafficking into DTC-harboring tissues. And since CD19 is not a breast cancer antigen, we need to identify DTC-relevant antigens and neo-antigens in breast cancer. Ghajar is working with other Artemis participants to identify viable TCR and CAR targets in breast cancer DTCs from patient bone marrow samples and aspirates.

Cell Free DNA Update

Ben Ho Park

One problem with the current treatment paradigm for breast cancer is that it leads to both the overtreatment and undertreatment of many people because we don't know how to distinguish, on an individual level, who has microscopic metastatic disease and needs additional therapy, and who does not. Measuring microscopic tumor burden could be a way to make clinical treatment decisions for breast cancer. The DNA mutations that make cancer are different from normal cells, and 100% specific to cancer.

All cells, both normal and cancerous, shed small DNA fragments into the blood called circulating cell-free DNA (ccfDNA or cfDNA). Circulating tumor DNA (ctDNA) refers to the DNA shed specifically by tumor cells. Plasma tumor DNA (ptDNA) is plasma-derived ctDNA. As a marker of whether micrometastatic disease is present, the tools exist for detecting ptDNA even though it has a short half-life that could decrease the sensitivity.

One area of research is the Droplet Digital PCR which was used in a pilot proof-of-principle study to detect ptDNA in women with a new diagnosis of early stage breast cancer.

The Pathologic Response Evaluation & Detection In Circulating Tumor-DNA (PREDICT-DNA) clinical trial is a collaboration between the Translational Breast Cancer Research Consortium and the Johns Hopkins Clinical Research Network looking at whether detection of ptDNA after neoadjuvant therapy is associated with pathologic response post-surgery. Although the trial is now closed to enrollment, it has accrued 228 patients with Stage II/III HER2+ or TNBC breast cancer, who are planning to have neoadjuvant treatment.

Participants discussed which mutations to look for in ptDNA that would cover the majority of patients, whether dormant or quiescent cells are shed at different rates than cycling cells, and scenarios that involve parallel evolution of cancer cells with truncal mutations that disseminate early from the primary tumor and represent only a minority of the primary tumor cells, or polyclonal tumors. Participants also discussed the ethical dilemma of women wanting a commercial lab test to confirm they do not have cancer, but there are not any data yet to support a conclusion that ten years of negative tests mean there is no disease.

PREVENTING METASTASIS WORKING GROUPS

Participants identified three topics on preventing lethal metastasis for further discussion. A summary from each group discussion is below.

1. TARGETABLE PROTEOMES/ CELL SURFACE

Rebecca Bish, Frank Calzone, Jay Debnath, Steve Elledge, Pat Haugen, Keith Knutson, Alana Welm

Group discussion focused on how to catalogue what is going on at the DTC cell surface level, and how to identify actionable targets to clear the DTC or keep it at bay, so it does not develop into a lethal metastatic outgrowth. If we could identify the simple unique characteristics of the cancer cell surface, then we could try to tether a foreign protein marker that could then be targeted by CAR T cells. This type of approach wouldn't require knowledge of the biology, and it would enable us to identify and find the DTCs in order to study them.

This group did not move forward with the recommendations.

2. TECHNOLOGY PROJECT

Danny Douek, Yaniv Erlich, Cyrus Ghajar, Judi Hirshfield-Bartek, Simon Knott, Chris Li, Kim Lyerly, Anne-Laure Papa, Ben Park, Fran Visco

Discussion first focused on two main topics. The group brainstormed different types of existing technologies/strategies that could be used to study dormant tumor cells waking up and becoming lethal metastasis. One point was that a pathologist can recognize a tumor cell just by looking at it. Can a technology be developed that does the same thing using physical properties and "appearance" to detect a tumor cell?

The second topic involved moving away from existing technologies to focusing on new technologies that would study dormant and lethal metastatic cells and what that technology would encompass. It was decided the focus of the first step of this project should be just to design a technology that can identify and monitor the process of metastasis and which metastases are lethal.

The main goal of a new technology is to identify quiescent tumor cell(s) and monitor the process of the cell(s) waking up and progressing to lethal metastasis. The new technology should be able to:

- Sense the tumor cells (e.g., physical properties, optical properties, specific cell surface chemistry, secreted chemistry, and/or changes in metabolism),
- Record events (genetically, optically) from the cell in real time and with spatial accuracy, and
- Report that information in a way that allows reconstruction of the spatial and temporal events.

Once DTCs are better characterized and understood, then this information can be given to the technology experts to design a technology able to recognize them.

NEXT STEPS

- Participants, via email, are to identify the details of key traits of technology needed for a white paper challenge.
- Simon Knott will look through one of the single-cell RNA seq datasets highlighted during his presentation to address the question of whether or not all DTCs will express EpCAM. He will also be able to determine whether all DTCs express an epithelial profile.
- Obtain data on whether the physical properties of DTCs might make them "invisible" to the immune system. Investigate the idea that DTC cell morphology can change in a way that makes T cells and other cells surveilling for it unable to recognize it.
- Once the DTCs are isolated, Danny Douek can help with Smart-Seq RNA sequencing, which he does routinely in this lab.
- Research additional people to consult with, to include a cell physicist, biophysicist, and someone involved in the "Star Wars" missile defense system to provide expertise on how the technology could relay information throughout the body.

3. INGREDIENTS OF SUCCESS FOR EQUILIBRIUM TO SUPPRESS DISEASE/ORGANS WITH DTCS

Stacie Canan, Mikala Egeblad, Silvia Formenti, Mary Helen Barcellos-Hoff, Brandy Heckman-Stoddard, Debbie Laxague, Michele Rakoff

The group discussed whether a diagnosis of invasive breast cancer implies dissemination. If breast cancer once detected has already disseminated, then why do some women develop clinical metastasis while others, despite disseminated cells, don't? If all breast cancer is disseminated on day 1, then the emergence of metastasis is determined by host/environmental factors including age, the type of immune system, stress, infections, and the environment (or diet).

On the other hand, if only a subset of cancer disseminates, then the weight of the host factors would be modeled differently. The host reaction might be the same regardless of the "seed," but perhaps with different success. There might be host genetics that result in biology able to suppress the biology of a "bad seed," such as centenarians with a BRCA mutation."

Participants discussed possible ways to learn from humans, and not just mouse models, perhaps by harnessing the big data movement. Examples included looking at breast cancer diagnosed within five years of pregnancy and further exploring the concept of "immune age."

During the large group discussion, it was noted that immune profiles need fresh blood samples in order to read the individual components. Equilibrium was also discussed in the context of many women having disseminated cells, but most are kept at bay, managed by the host, so they do not become clinically evident and eventually lethal metastasis.

We need to identify host characteristics associated with distant recurrence and death, along with a "low risk" host profile. We need to develop a tool comparable to the polygenic risk score, but for breast cancer death, and enriched with additional information about the host. We want to define associations of host factors to identify modifiable host factors.

Two-prong approach:

- Retrospective cohort with a case-control design
  - Cases would be those patients with early distant recurrence and death, and controls those who had lymph node-positive breast cancer with no recurrence after 15 years

- Use samples from Breast Cancer Cohort Consortium and available GWAS, cytokine, and longitudinal CBC with differential data from analyses already conducted

- Prospective cohort with serial PBMC (peripheral blood mononuclear cells) samples
  - Patients at primary diagnosis
  - Nest within a new NCI adjuvant trial to take advantage of study infrastructure
  - Additional blood draw to enable IMM-AGE prospective analysis

Next year deliverable: preliminary "host profile" associated with breast cancer death (distant metastasis).

4. BOOSTING THE IMMUNE SYSTEM (NEW SUBGROUP)

Rebecca Bish, Frank Calzone, Steve Elledge, Pat Haugen, Keith Knutson, Ben Park, Alana Welm

This small group topic came out of the morning brainstorming session and focused on transferring antibodies from immunotherapy strong responders to non-responding patients to boost their immune system.

NEXT STEPS/PROCESS

- Identify long-term survivors, and profile the patients
  - Not just long-term survivors, but those with evidence of disease or exceptional immune-based responders
- Determine whether selected population has antibodies that bind tumors, and conduct functional assays (serum/plasma)
  - Flow cytometry (FACS) for selection of responders
    - Tissue samples or cancer cells? Include microenvironment?
    - Phage display linear epitopes
    - ORF display conformational epitopes
  - Doublet cell FACS tumor binding to select patient samples to take forward
  - NK cell assay; xenograft plus antibody injection or immune repertoire
  - Identify antibodies able to kill cells
    - In vitro cell killing assay
    - In vivo xenograft, or mouse model with human immune system
- Clone the antibodies

PREVENTING METASTASIS – WILD IDEAS, NEW IDEAS

As a final activity for the Preventing Metastasis meeting, participants paired up and were encouraged to think big and bold, and to put aside DTCs, new targets and drugs. The group brainstormed ideas and began to develop different approaches, such as disrupting CTCs, preserving and rejuvenating the immune system and exceptional responders, among other areas. This discussion will inform future Artemis work.

IV. ARTEMIS PROJECT ON PRIMARY PREVENTION

BACKGROUND PRESENTATION

Preventive Breast Cancer Vaccine: Update

Keith Knutson

Knutson updated the group on the preventive vaccine project. The goal of the preventive breast cancer vaccine is to generate an adaptive memory immune response that persists for many years.

Since last year's Artemis meeting, we have been preparing an Investigational New Drug (IND) application to submit to the FDA. The IND will include preclinical safety and immunologic efficacy data, a Phase 1 trial design, vaccine production details, and the development of immune response/surrogate assays.

Sara Chumsri is leading the Phase 1 study at the Mayo Clinic Cancer Center. The clinical protocol has been developed for a Phase 1 safety trial in patients with low-volume stable metastatic breast

cancer. The vaccine will be DNA-based using a prime-boost strategy. The initial prime will be with plasmid DNA followed 30 days later with virus-encoded antigen. Twenty-five patients will be evaluated for both safety and immunogenicity. The feasibility assessment was completed the week prior to the Artemis meeting, and we have received approval from the scientific advisory committee.

The vaccine product has two constructs with the plasmid and MVA (modified vaccinia virus Ankara): one with HER-2/neu and MUC1, and the other with Mammaglobin-A, Survivin, hTERT, and MAGEA3.

Participants discussed where and how the vaccine should best be injected to generate T cells that will home back into the tissue. Although an intramuscular injection is technically easier than an intradermal injection, there is no evidence that one is better than the other. Another suggestion was to inject the vaccine directly into the breast lymph system, and it was noted that the infectious disease field is moving toward intravenous injections because it seems to work well for intra-tissue immune responses. There is evidence that memory T cells expand in the nearest draining lymph node following vaccination in the upper half of the body. Other issues raised included whether or not there would be tolerance, and how the immune response is going to be strong enough to eliminate cancer cells, but not other cells.

PRIMARY PREVENTION WORK GROUPS

Participants identified three topics on primary prevention for further in-depth discussion. A summary from each group discussion is provided below.

1. Clinical Trials/Immune Monitoring

Rebecca Bish, Stacie Canan, Danny Douek, Silvia Formenti, Brandy Heckman-Stoddard, Keith Knutson, Debbie Laxague

The primary topic of discussion related to advancing the prevention vaccine to phase 1 and then phase 2 clinical trial testing, including study populations, designs, and outcomes of interest. Discussion also involved what a subsequent phase 3 trial design would look like.

The phase 1 trial is primarily focused on safety. Each of the plasmid components of the vaccine have already been tested and have been shown to be safe, but there is concern about delivering the antigens all at once. That would be new. The FDA specified that the population to be included in phase 1 should be women with stage 4 metastatic disease with small volume, low disease burden. Participants will be pre-menopausal women, not pregnant, and on birth control. For phase 1, the FDA is only concerned about safety with a required follow-up time of 30 days from the last booster. However, Knutson hopes to follow the participants for a couple of years. Immunogenicity outcomes were also suggested for the phase 1 trial, which will require more than 30 days of follow-up.

A phase 2 trial will be looking for a physiologic change in mucosal immunity, including generation of T cells. The group agreed that at least six months of follow-up data were needed in the phase 2 study, with measurements at 1, 3, and 6 months. Participants discussed potential patient accrual issues and suggested a study population of neo-adjuvant triple-negative breast cancer (TNBC) patients or BRCA1/2 carriers. Ultimately, the vaccine will likely be used in the population of women past child-bearing age, approximately 45 years old, and at high risk for breast cancer.

Another suggestion was to run a study in parallel with the phase 1 trial among patients undergoing prophylactic mastectomy to characterize the mucosal breast immunity and its variability. This is a growing population of patients that could be immunized eight weeks prior to mastectomy, and then their removed breast tissue could be used to look for correlates to apply in future clinical trials.

NEXT STEPS:

Phase 1 clinical trial (n=25 patients)

- Stage 4 breast cancer patients with minimum disease burden (study population specifically required by the FDA)
- Vaccine cost will be about $600-700k, although it does not have to be GMP-produced
- Safety endpoints: measure antibody responses using developed immune assays

Knutson is running a parallel study to look at tissue samples in women to characterize the tissue landscape in normal tissue, tissue with atypical hyperplasia, and diseased breast tissue using samples from the Komen tissue bank. Participants suggested gathering similar data over the next 12-18 months among the same population to be included in the phase 2 study, women undergoing prophylactic mastectomy. These samples could be compared to determine whether women at high-risk of breast cancer have a different mucosal immunology.

Phase 2 clinical trial (n=100 patients)

- Women at high-risk of developing breast cancer and undergoing bilateral prophylactic mastectomy (women with two breasts)
- Randomization (1:1) to vaccine or no vaccine
- Biopsy, vaccinate on one side, collect tissue specimens following surgery
- **Physiologic endpoint:** measure local immunogenicity in both breasts to assess whether vaccination is needed on both sides of the body (in both arms), and to identify tissue markers for use in a phase 3 efficacy trial

Phase 3 clinical trial (n=5,000-10,000 patients over 10 years)

- Women at high-risk for developing invasive breast cancer (diagnosed with atypical hyperplasia or LCIS, treated DCIS, or genetic BRCA carrier) who don't want medical therapy (self-selected), and who have at least one breast at risk
- Women on tamoxifen, raloxifene, or aromatase inhibitors would be excluded

NEXT 12-18 MONTHS:

- Complete IND and submit to FDA
- Enroll patients in phase 1 clinical trial
- Clinical study of breast mucosal immunity to understand variability
- Define and optimize phase 2 clinical trial endpoint
- Produce vaccine
- Hire a vaccine production project manager

2. Predator/Prey: Operation Prairie Justice

Frank Calzone, Jay Debnath, Cyrus Ghajar, Pat Haugen, Ben Ho Park, Kim Lyerly

In the non-cancerous breast, the breast epithelium is polarized and expresses apical proteins, which are exposed only to the lumen of the ducts/acini. During breast cancer, cells escape the polarized epithelium, which results in their apical proteins being exposed to the tissue rather than being protected in the lumen. This breakout group discussed leveraging this depolarization and exposure of apical proteins in the tissue as a novel way for T cells to recognize and eliminate breast cancer cells.

The group established a timeline and a more refined experimental approach to begin to test the hypothesis that CAR T cells directed to an apical protein could be used to eliminate breast cancer cells.

- **Months 0-6:** 3D cell culture models to test whether CAR T cells directed against apical proteins can selectively kill breast cancer cells. Cell lines suggested were: S1, MCF10A, primary tumor organoids, and MDCK cells.
- **Months 0-12:** Transgenic development with CD19 apically localized to epithelial cells. It was suggested that these mice could be generated using in utero transduction techniques to express the protein in the whole mammary epithelial tree or CRISPR for whole body expression.
- **Months 6-18:** Utilize 3D organoid models with the addition of MMP-9 to identify unique basement membrane fragments that could be used to localize CAR T cells to the breast. Mass spec would be used to identify membrane fragments.
- **Months 9-18:** Test CD19 CAR T cells in the transgenic mice developed above. Determine whether the CAR T cells attack only depolarized epithelial cells. Unfortunately, the model suggested above (Months 0-12) may not be appropriate to assess toxicity because every epithelial cell (in mammary tree or body) would be expressing the protein.
- **Months 18-24:** Dual-specific CAR T cell development to localize the CAR T cells to the breast and kill cells with exposed apical proteins.
- **Months 15-24:** MTMV-HER2delta16 mice expressing apical antigen of interest to test CAR T cells.
- **Months 24+:** Testing

3. New Ideas for Primary Prevention

Steve Elledge, Judi Hirshfield-Bartek, Simon Knott, Chris Li, Anne-Laure Papa, Alana Welm

This was a brainstorming session to come up with various approaches to primary prevention. There were no restrictions placed on the discussion. All ideas were welcome. The ideas presented included keeping anti-estrogen therapy local to the breast. Selective estrogen receptor modulators (SERMs), like tamoxifen and raloxifene, are based on the idea that regulating hormones will eliminate breast cancer. And while SERMs have been shown to reduce the incidence of breast cancer, although

there was debate about this, there are significant side effects and no effect on breast cancer mortality has been seen. Developing a new technology to enable intraductal administration would keep the anti-estrogen therapy local and avoid the systemic side effects.

The group then discussed differentiation therapy as a way to block cell proliferation in the breast. Differentiation therapy is standard for leukemia, and the protective effects of pregnancy for breast cancer are thought to be due at least in part to the differentiation of stem cells. We could screen for compounds that would force breast stem cells to differentiate, and then deliver them intraductally (locally) to avoid systemic effects. Participants discussed the consequences of eliminating all the stem cells in the breast.

Another idea, based on Dr. Sue Love's work on the breast microbiome, was to engineer an estrogen "sink" using bacteria that like to live in the breast. The bacteria could be engineered to bind and soak up estrogen or modify or destroy it, resulting in local modulation of proliferative signals. Participants discussed enzymes that would destroy or convert estrogen into other forms, but concerns were raised about the idea of estrogen deprivation therapy, which has been shown to have lots of side effects. Another concern raised was that the bacteria may not be in the same locations as estrogen.

All of these new ideas hinge on the assumption that stem cells respond to estrogen. How can we verify that ductal breast stem cells are causing cancer?

NEXT STEPS:

Differentiation therapy: Consider a local therapy to induce breast cells to differentiate, or a systemic therapy if breast-specific

1) **Normal breast organoids:** Human organoids could be screened in a high-throughput format. Organoids are a mixture of different cell types grown in a ductal structure that allows for studying cell-cell interactions and breast cell growth/differentiation.

a. Chemical screens with known bioactive libraries (plus hormones we know are important for differentiation as a positive control) could be performed to look for things to induce differentiation and that target candidate pathways

b. CRISPR screens for gain or loss of function

c. Determine what kind of assay/reporter could be used

i. A stem cell reporter that reports loss of "stemness" would be a good approach to account for different lineages and different pathways to differentiation

ii. Is there a 2D reporter line for genome-wide screening?

iii. Using a cell line might be faster than organoids, but it would not necessarily be as relevant.

2) **Proof-of-concept in mouse model:** If you eliminate or differentiate mouse stem cells in vivo, would you prevent the mouse from developing breast cancer?

Months 12-18:

- Chemical screens with hormones, and the mouse proof-of-concept experiments could be run in parallel
- Identify lead candidates and plan how to move forward
- Need expertise in endocrinology, mammary stem cell biology

Breast microbiome: Bacterial sink for estrogen and/or progesterone

- Engineer commensal breast bacteria to bind estrogen and/or progesterone in the breast, or to express anti-E2/P2 nanobodies or SERDs (selective estrogen receptor degrader) locally – need endocrinology expert to weigh in
- Engineer bacteriophages to infect endogenous bacteria locally
- Rodent model testing might not be appropriate due to microbiome differences, and so it would need to be tested in women
  - Initial testing could be in women prior to mastectomy
  - Biopsy pre-surgery, surgery, assess surgical tissue specimens for ER responses before and after treatment

APPLYING NEW TECHNOLOGIES TO PAST ARTEMIS PLANS

At the beginning, the Artemis Project provided seed grants to identify antigens for the first version of the breast cancer preventive vaccine from The Cancer Genome Atlas (TCGA). The selection of antigens occurred in 2015. Now, in 2019, have the technologies changed enough for it to be worth going back and using currently available

technology to do another round of "antigen searching"? Also, previously, a viral cause of breast cancer wasn't identified through another Artemis seed grant. Does the technology exist now to re-visit the virus question?

The group of participants discussed high resolution technologies, the potential role of artificial intelligence, the microbiome, and new sequencing and taking a new look at data related to the initial DCIS seed grant.

NEXT STEPS:

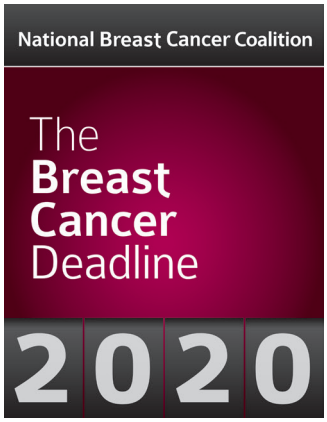
- Simon Knott to send data to Danny Douek to run through his microbiome pipeline
- Advocates to look again at available matched pair data?

V. CONCLUSION

The Artemis Project has produced a number of effective collaborations among diverse researchers and advocates. The Project participants continue to focus on primary prevention and the prevention of metastasis. Important progress has been made in the critical activities needed to develop and test a preventive vaccine for breast cancer and to understand the process of metastasis and how to stop it. A strategic plan for the development of a preventive vaccine was launched in 2011 and is being implemented through the Artemis Project® for a Preventive Breast Cancer Vaccine.

Following positive discussions with FDA in 2018, the vaccine development team has been preparing an Investigational New Drug (IND) application to submit to the FDA at the end of 2019. The IND will include preclinical safety and immunologic efficacy data, a Phase 1 trial design, vaccine production details, and the development of immune response/surrogate assays. Dr. Sara Chumsri will serve as the primary clinical investigator for the Phase 1 study at the Mayo Clinic Cancer Center.

The group also discussed how data would be best used to identify targets for preventing lethal disease and risk reduction. In addition to these directed activities, participants in the Artemis Project are continuously reevaluating the state of the sciences to ensure that alternatives, or additional opportunities to prevent breast cancer and end deaths are being considered, and appropriately incorporated into the goals of the Artemis Project.



National Breast Cancer Coalition
1010 Vermont Ave., NW, Suite 900
Washington, DC 20005

BreastCancerDeadline2020.org